

SUPPLEMENTAL FIGURES

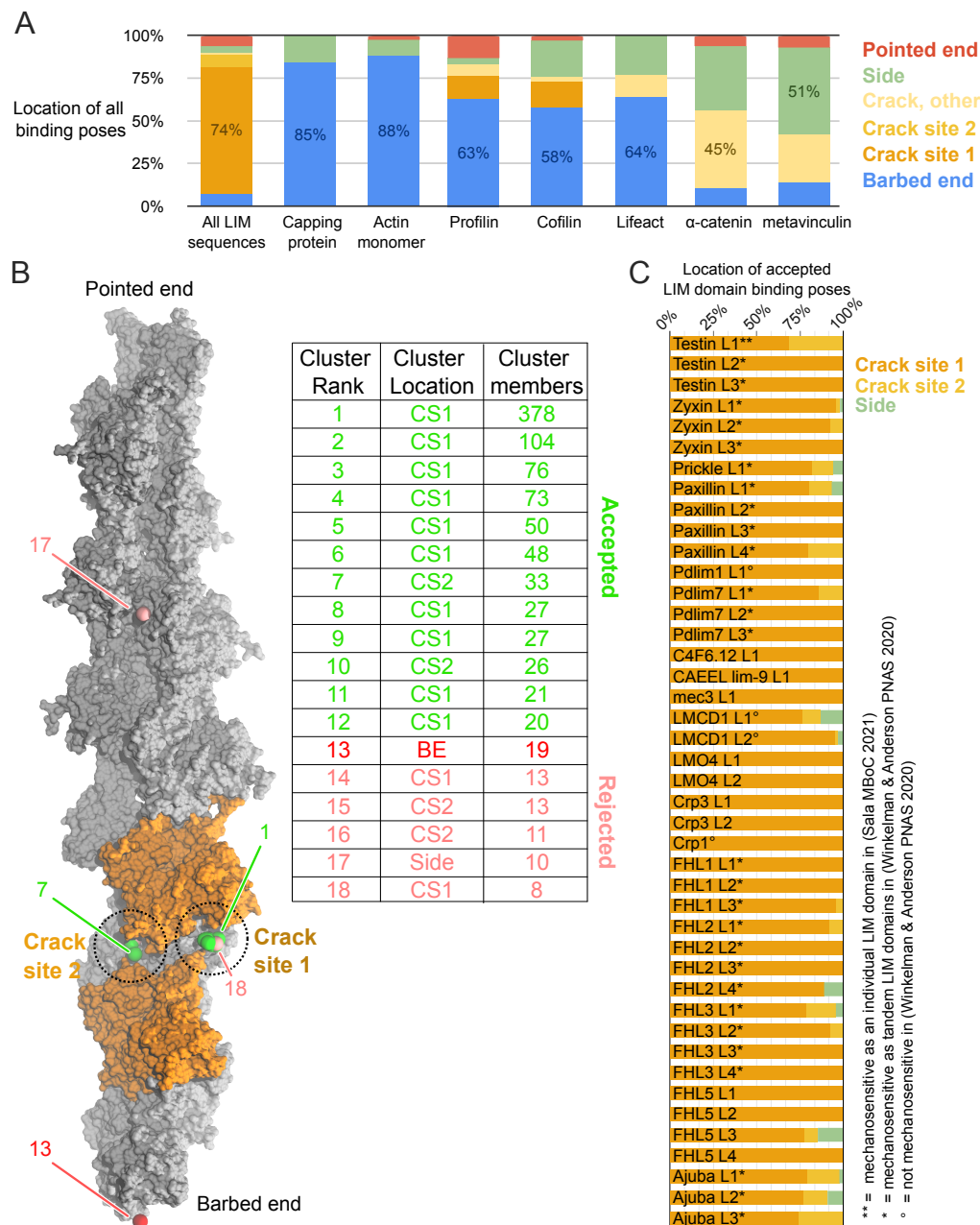


Figure S1. Docking simulations of ABPs to a cracked actin filament.

(A) Location of all docking poses on a cracked actin 13-mer receptor. Each column corresponds to the indicated ligand. All 43 LIM domain sequences are shown at left as an aggregated dataset. The most likely binding location for each ligand is labeled as a percentage on the bar. Note that LIM domains have a striking preference for the cracked interface.

(B) Illustration of docking simulation processing. (Left) Space-filling model of the actin 13-mer receptor with subunits at the cracked interface highlighted in orange. Spheres represent the center of mass of each binding cluster output by ClusPro2.0 for a single LIM domain sequence. Several spheres are labeled with their ranking by cluster size. Color corresponds to acceptance/rejection.

(Right) Table of all binding poses. Poses with cluster size larger than the highest-ranked end-binding pose (red) are accepted (green) while others are rejected (pink). CS1/CS2 = crack site 1/2, BE = barbed end.

(C) Location of all accepted binding poses for each LIM domain sequence. In total, ~93% of accepted binding is at CS1, ~5% is at CS2, and <2% is on the filament side, not at the crack site.

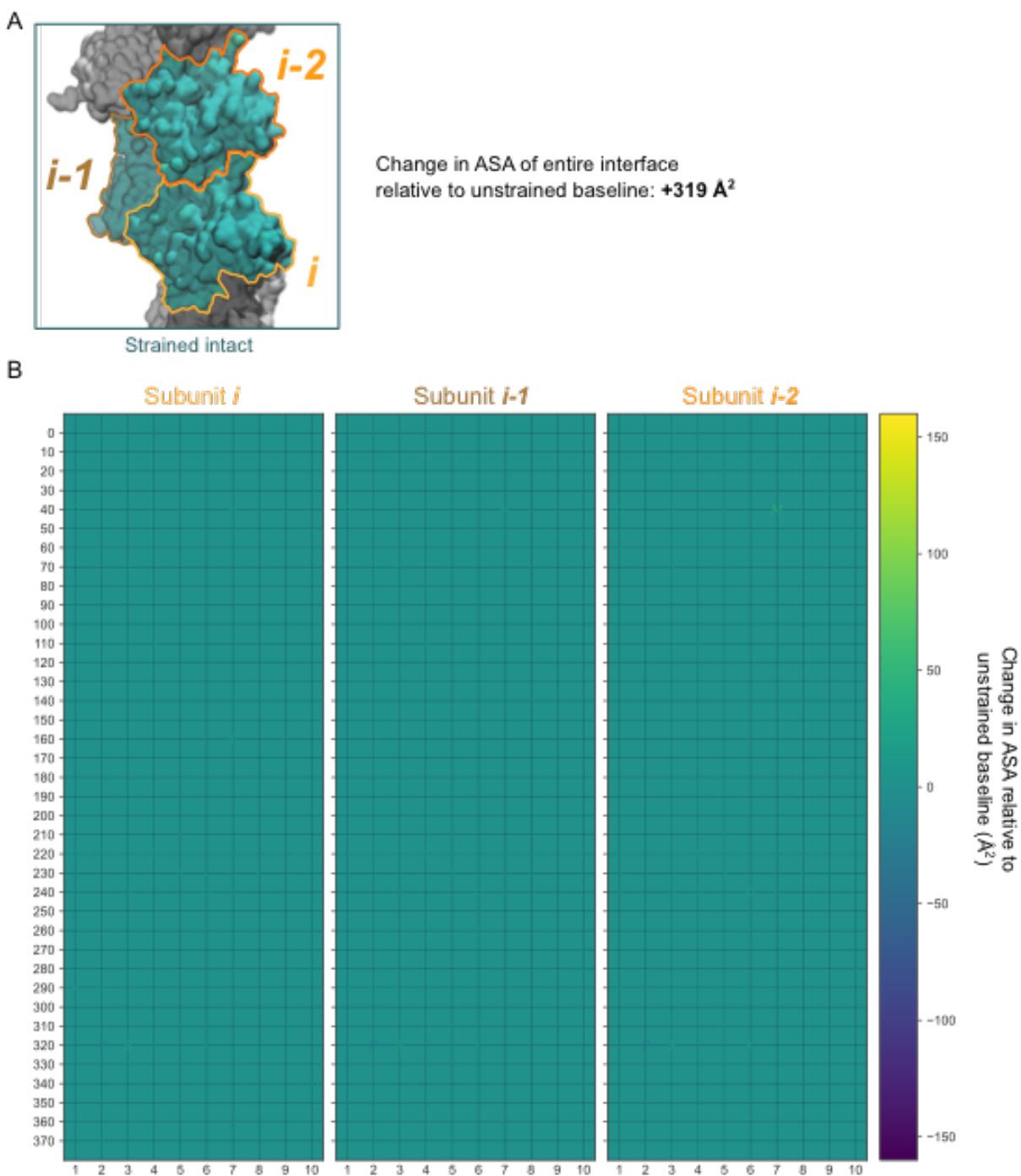


Figure S2. Strained-intact interfaces present a similar binding surface to interfaces in unstrained actin filaments.

(A) Space-filling representation of a representative strained-intact interface. Amino acid color corresponds to change in accessible surface area (ASA) relative to unstrained actin filaments according to the color bar in (B).

(B) Three grids list every amino acid in subunits *i*, *i-1*, and *i-2* at a strained-intact interface. The size and color of each amino acid is scaled based on the change in ASA. Given that all residues

in strained-intact filaments maintain roughly the same ASA of unstrained filaments, these grids appear empty. The ASA probe radius is 4 Å. $n = 22$ simulations.

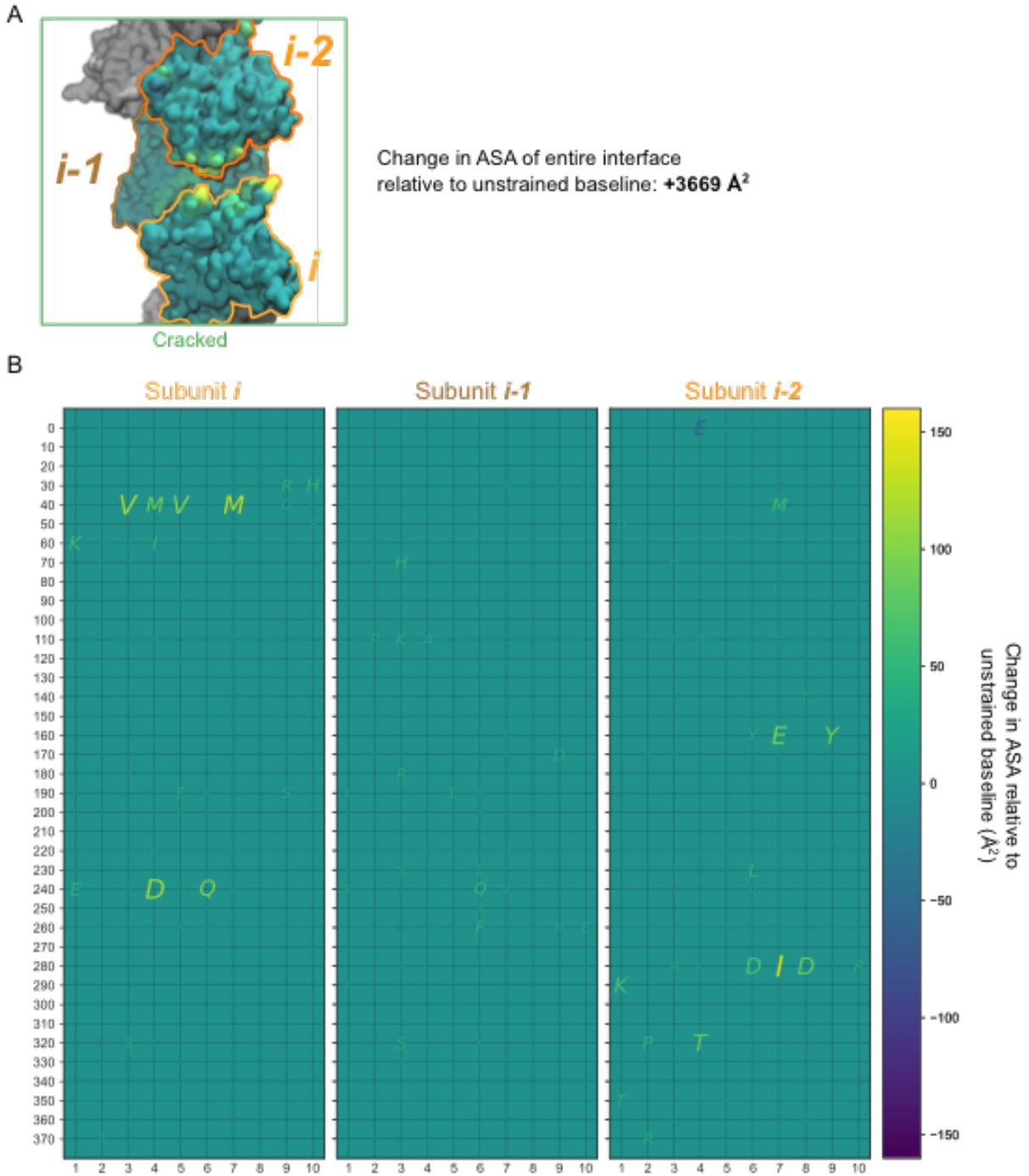


Figure S3. Cracked interfaces present a unique strain-induced binding surface.

(A) Space-filling representation of a representative cracked interface. Amino acid color corresponds to change in accessible surface area (ASA) relative to unstrained actin filaments according to the color bar in (B).

(B) Three grids list every amino acid in subunits *i*, *i-1*, and *i-2* at a cracked interface. The size and color of each amino acid is scaled based on the change in ASA. The ASA probe radius is 4 Å. $n = 45$ simulations.

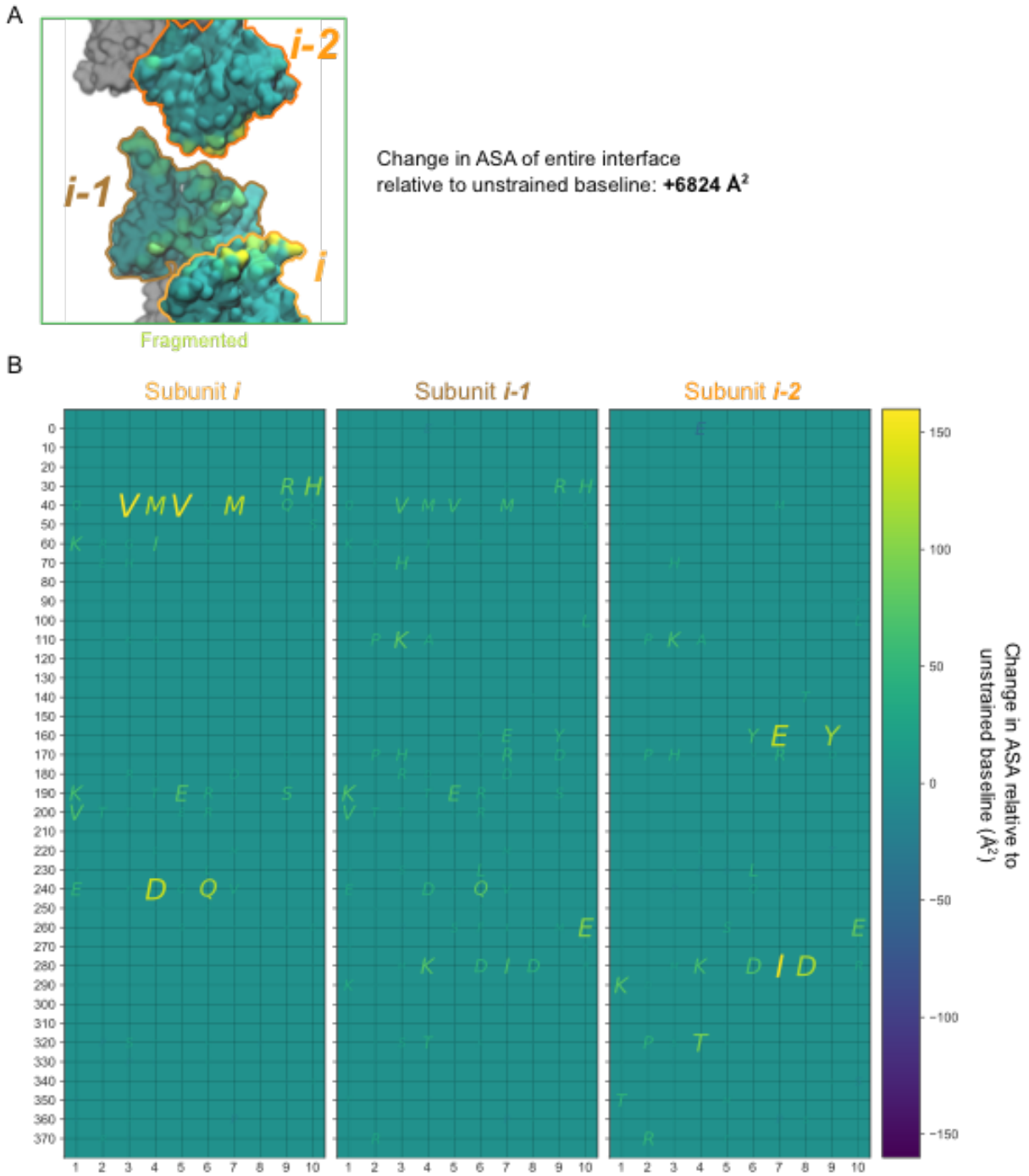


Figure S4. Filament fragmentation exposes typically-buried amino acids.

(A) Space-filling representation of a representative cracked interface. Amino acid color corresponds to change in accessible surface area (ASA) relative to unstrained actin filaments according to the color bar in (B).

(B) Three grids list every amino acid in subunits *i*, *i-1*, and *i-2* at a fragmented interface. The size and color of each amino acid is scaled based on the change in ASA. The ASA probe radius is 4 Å. $n = 45$ simulations.

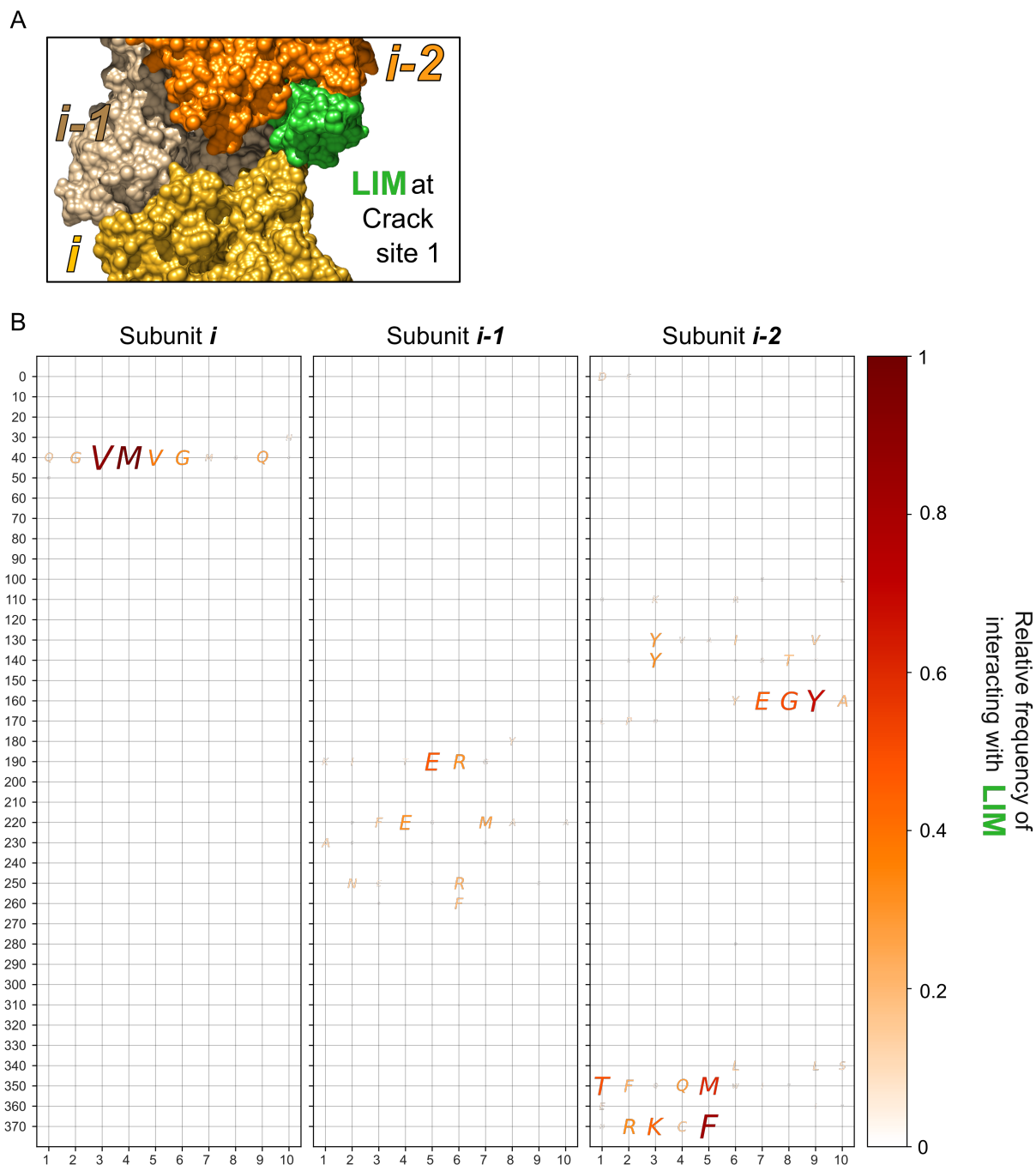


Figure S5. LIM domains bound to crack site 1 interact with residues that become exposed at cracked interfaces.

(A) Space-filling representation of a LIM domain (green) bound to the cracked interface at crack site 1.

(B) Three grids list every amino acid in subunits *i*, *i-1*, and *i-2* at the cracked interface. The size and color of each amino acid is scaled based on its relative frequency of interacting with LIM bound to crack site 1 across all accepted binding poses of all 43 LIM domain sequences. Contact was calculated using a 5 Å cutoff.

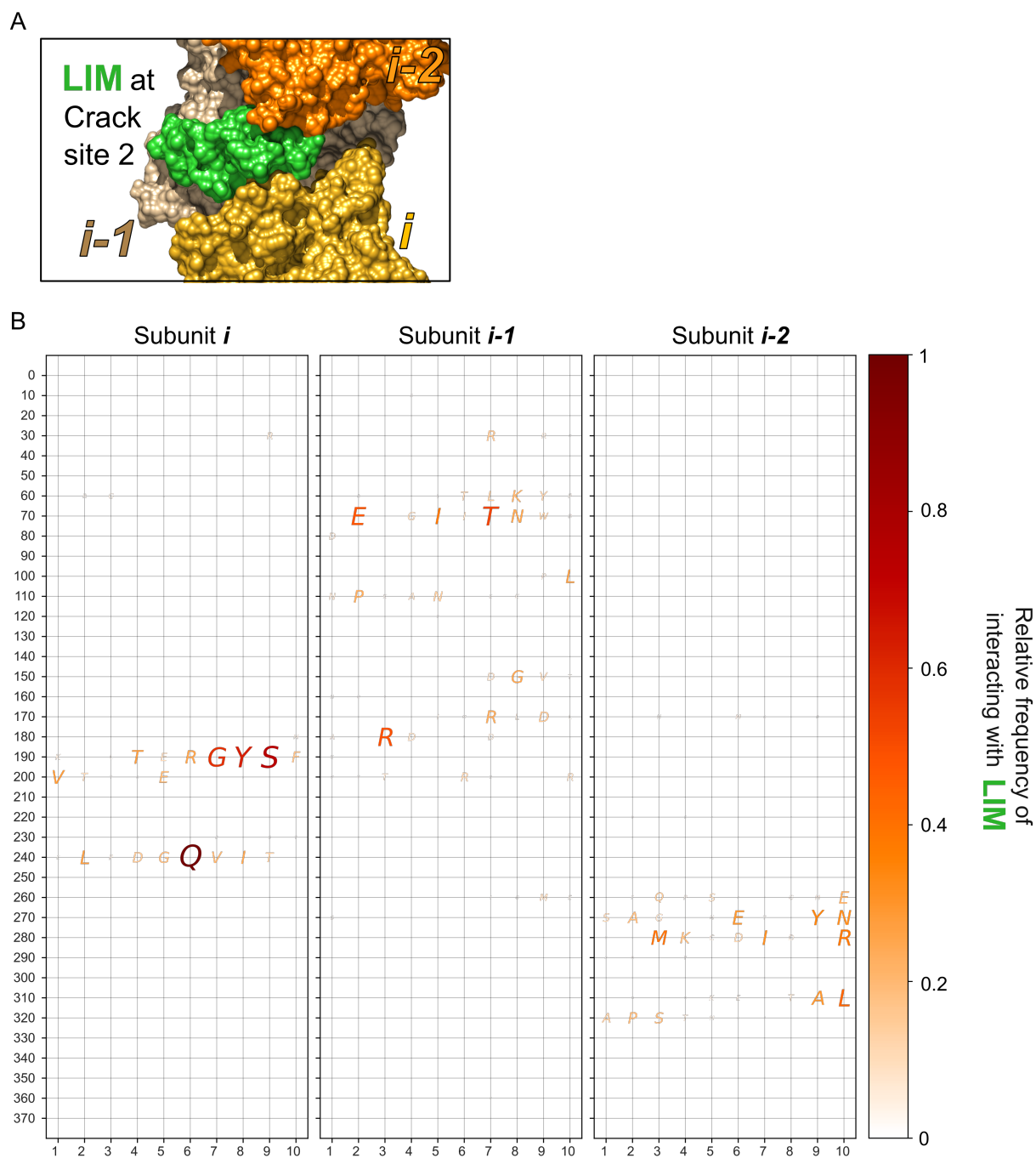


Figure S6. LIM domains bound to crack site 2 interact with residues that become exposed at cracked interfaces.

(A) Space-filling representation of a LIM domain (green) bound to the cracked interface at crack site 2.

(B) Three grids list every amino acid in subunits *i*, *i-1*, and *i-2* at the cracked interface. The size and color of each amino acid is scaled based on its relative frequency of interacting with LIM bound to crack site 2 across all accepted binding poses of all 43 LIM domain sequences. Contact was calculated using a 5 Å cutoff.

Movie S1. Actin filaments crack before they fragment. An actin filament under 500 pN tension initially maintains all connections between subunits. Eventually a longitudinal interface on one protofilament loses contact, resulting in a metastable cracked conformation. Under continued strain, the opposite protofilament breaks and the filament fully fragments. The duration is 123 ns and frames are smoothed using a 3-ns window to clarify dominant motions.

Movie S2. The flip of Y169 (black) of subunit i-2 (yellow) away from the D-loop of subunit i (orange) precedes the first longitudinal interface breaking (crack formation). Frames are smoothed using a 3-ns window to clarify dominant motions. The duration is 100 ns, and the filament is subjected to a 550 pN tension.

Movie S3. Crack site 2 becomes exposed before fragmentation of an already-cracked filament stabilized by LIM domains. The last 170 ns are shown of a 2.43 μ s simulation of an actin filament with two LIM domains (green) bound to crack site 1 and 2 at the interface of subunits i (yellow) and i-2 (orange). Before fragmentation occurs, crack site 2 becomes exposed on the neighboring interface of the opposite protofilament strand between subunits i-1 (tan) and i-3 (pink, *top*). This may allow an unbound tandem LIM domain (not shown) to bind cracks on both protofilament strands simultaneously. Frames are smoothed using a 1-ns window to clarify dominant motions. The filament is subjected to 250 pN tension.

SUPPLEMENTAL TABLE

Table S1: Simulations performed in this study						
Simulation number	Initial structure	Number of actin subunits	Tension (pN)	Replicate number	Duration (ns)	Final state
1	6DJO	13	0	1	373	Intact
2	6DJO	13	0	2	988	Intact
3	6DJO	13	0	3	580	Intact
4	6DJO	13	200	1	1,091	Intact
5	6DJO	13	200	2	300	Intact
6	6DJO	13	400	1	810	Intact
7	6DJO	13	400	2	400	Intact
8	6DJO	13	500	1	91	Fragment
9	6DJO	13	500	2	123	Fragment
10	6DJO	13	500	3	340	Fragment
11	6DJO	13	500	4	307	Fragment
12	6DJO	13	500	5	251	Fragment

13	6DJO	13	500	6	510	Fragment
14	6DJO	13	500	7	180	Fragment
15	6DJO	13	500	8	110	Fragment
16	6DJO	13	550	1	200	Fragment
17	6DJO	13	550	2	100	Fragment
18	6DJO	13	550	3	80	Fragment
19	6DJO	13	550	4	194	Fragment
20	6DJO	13	550	5	72	Fragment
21	6DJO	13	600	1	40	Fragment
22	6DJO	13	600	2	82	Fragment
23	6DJO	13	600	3	87	Fragment
24	6DJO	13	600	4	30	Fragment
25	6DJO	13	600	5	40	Fragment
26	Cracked	13	0	1	57	Intact
27	Cracked	13	0	2	142	Intact
28	Cracked	13	0	3	101	Intact
29	Cracked	13	300	1	171	Fragment
30	Cracked	13	300	2	141	Fragment
31	Cracked	13	300	3	55	Fragment
32	Cracked	13	300	4	358	Fragment
33	Cracked	13	300	5	60	Fragment
34	Cracked	13	350	1	30	Fragment
35	Cracked	13	350	2	70	Fragment
36	Cracked	13	350	3	70	Fragment
37	Cracked	13	350	4	100	Fragment
38	Cracked	13	400	1	40	Fragment
39	Cracked	13	400	2	20	Fragment
40	Cracked	13	400	3	27	Fragment
41	Cracked	13	400	4	40	Fragment
42	Cracked	13	400	5	40	Fragment
43	Cracked	13	450	1	20	Fragment
44	Cracked	13	450	2	30	Fragment
45	Cracked	13	450	3	20	Fragment

47	Cracked	13	500	1	30	Fragment
47	Cracked	13	500	2	18	Fragment
48	Cracked	13	550	1	20	Fragment
49	Cracked	13	550	2	20	Fragment
50	Cracked	13	550	3	20	Fragment
51	Cracked	13	600	1	11	Fragment
52	Cracked	13	600	2	10	Fragment
53	Cracked	13	600	3	10	Fragment
54	Strained-intact	13	500	1	64	Fragment
55	Strained-intact	13	550	1	30	Fragment
56	Cracked	7	250	1	70	Fragment
57	Cracked	7	250	2	20	Fragment
58	Cracked	7	250	3	50	Fragment
59	Cracked	7	250	4	50	Fragment
60	Cracked	7	250	5	50	Fragment
61	Cracked	7	500	1	11	Fragment
62	Cracked	7	500	2	10	Fragment
63	Cracked	7	500	3	5	Fragment
64	Cracked+LIM ¹	7	250	1	1,479	Fragment
65	Cracked+LIM	7	250	2	2,430	Fragment
66	Cracked+LIM	7	250	3	370	Fragment
67	Cracked+LIM	7	500	1	21	Fragment
68	Cracked+LIM	7	500	2	15	Fragment
69	Cracked+LIM	7	500	3	15	Fragment
Total	—	—	—	—	13,800	—

1. System composed of a cracked actin 7-mer with two copies of the first LIM domain of testin bound to crack sites 1 and 2.